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An effective and sensitive stability-indicating chromatographic approach based on HPLC for silodosin assay

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Abstract

Background: A stability-indicating reversed-phase high-performance liquid chromatography (HPLC) method with a high sensitivity was developed for the determination of silodosin (SIL) in the presence of hydrochlorothiazide (HCT) as an internal standard.

Methods: Chromatographic separation of SIL and IS were successfully achieved on an Agilent ZORBAX CN column with an isocratic mobile phase composed of a mixture of methanol:acetonitrile:ammonium acetate (pH 4.0; 0.015 M) (40:30:30, v/v/v) at a flow rate of 1.3 mL min⁻¹. The drugs were quantified using a photodiode array detector set at a wavelength of 270 nm. The reversed-phase HPLC method has been validated as per International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines to determine SIL in pharmaceutical dosage form.

Results: The proposed method showed a good linearity in the concentration range of 4.0–600.0 µM with a lower detection limit of 85.0 nM under optimized conditions. The statistical performance of the fully validated HPLC method was compared to our developed sensitive spectrofluorimetric method, and the performance results of the proposed HPLC method were considerably satisfactory. The validated method was successfully applied to quantify the SIL in capsules, and the corresponding recovery value was found to be 99.5 %.

Conclusions: The validated HPLC method may be a promising alternative analytical tool for routine analysis of SIL in pharmaceutical samples.

Keywords: Silodosin, HPLC, Spectrofluorimetry, Determination, Validation

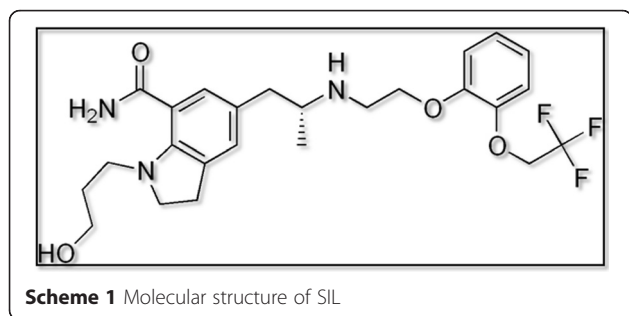
Background

Silodosin (SIL) is a new selective α_1 -adrenoreceptor antagonist in alpha-blocker class with a high pharmacologic selectivity as shown in Scheme 1 (Sweetman 2009). α_1 -adrenoreceptor antagonists were widely used in the treatment of lower urinary tract symptoms resulting from benign prostatic hyperplasia (BPH). BPH is a common disorder of the urogenital tract for at least 50 % of men aged over 50 years (Goi et al. 2015). At this point, SIL has been proven to advanced relieve the muscles in the prostate by reducing lower urinary tract symptoms related to BPH (Goi et al. 2015; Yoshida et al. 2007). Therefore, a reliable and precise determination method

is highly important for the analysis of SIL in pharmaceutical or clinical samples.

The literature revealed that various analytical methods have been reported to determine the SIL in pharmaceutical or clinical samples including UV spectrophotometry (Jahan and Malipatil 2014b), spectrofluorimetry (Bhamre and Rajput 2014), high-performance liquid chromatography (HPLC) (Jahan and Malipatil 2014a; Aneesh and Rajasekaran 2012; Vali et al. 2012), high-performance thin-layer chromatography (Sayana et al. 2012), ultra high-performance liquid chromatography (UHPLC) (Shaik et al. 2014; Prasad et al. 2012) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Zhao et al. 2009) and electrochemical sensing (Er et al. 2015) methods. Recently, the authors have attached great importance develop the sensitive and reliable analytical methods for

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determination of biological and drug molecules at nano-molar levels. In this point, we thought that an effective and highly sensitive analytical tool such as HPLC is required with a short response time for SIL assay. In addition, the spectrophotometric approach is also preferred especially in the detection of drug molecules due to its many advantages such as cost effectiveness, easy operation, high sensitivity and repeatability, rapid response time and low detection limit (Tekkeli and Önal 2011; Walash et al. 2013). The present study has indicated accurate and efficient analytical methods based on reversed-phase HPLC and spectrofluorimetry for the determination of SIL in a pharmaceutical sample.

Methods

Chemicals and reagents

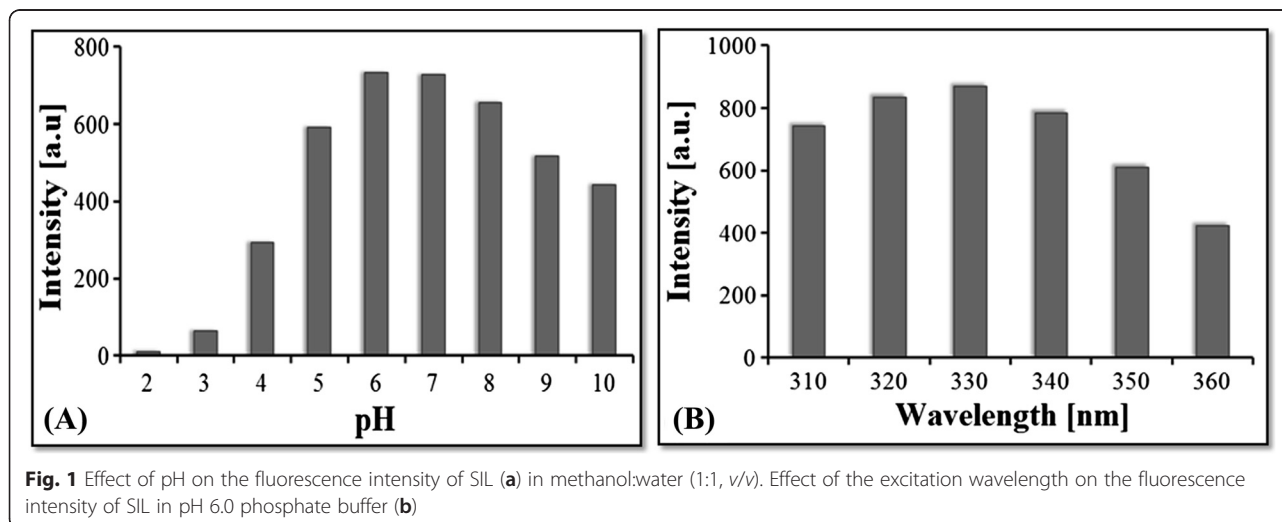
SIL powder was gifted from Recordati Pharm. Company. Urorec[®] (containing 8.0 mg SIL per capsule) capsules were purchased from a local market in Ankara, Turkey. The internal standard (IS) was used as a hydrochlorothiazide (HCT) in HPLC measurements. The methanol and acetonitrile solutions for HPLC studies were purchased

from J.T. Baker (Phillipsburg, NJ) with HPLC grade. The other chemicals and reagents were purchased from Sigma-Aldrich Company (Germany) and were of analytical grade. All solutions and mobile phase were prepared with ultrapure water using Waters Milli-Q Plus purification system.

Instrumentation and conditions

HPLC analyses were performed by an Agilent 1100 series LC system (Agilent Technologies, Wilmington, USA) equipped with an Agilent series G-1315B diode-array detector (DAD), G-1313A ALS autosampler, G1311A Quat pump and G1379A degasser. Chromatographic separation was performed on a Agilent ZORBAX CN column (reversed-phase) (150 mm × 4.6 mm, 5 μm) in isocratic mode. Data were collected and processed by the use of Agilent ChemStation. The mobile phase consisted of a mixture of aqueous 0.015 M ammonium acetate (pH 4.0):methanol:acetonitrile in the ratio (30:40:30, v/v/v). The pH of the ammonium acetate was adjusted to 4.0 by addition of NaOH and HCl, and the mixture was pumped at 25 °C with a flow rate of 1.3 mL min⁻¹. The detection was achieved at 270 nm, and the injection volume was 10 μL. The mobilephase mixture was filtered through a 0.45μm membrane filter (Millipore, Bedford, MA) and degassed under ultrasonic bath before HPLC analysis. The quantification of SIL was based on peak area ratio using IS.

Fluorescence spectra were measured by Agilent Cary Eclipse spectrofluorometer (CA, USA) equipped with a Xenon flash lamp. The slit widths for excitation and emission monochromators were fixed at 10 nm. All measurements were performed in a 1.0cm quartz cell at room temperature (25 °C).



Analytical procedure

Preparation of standard solutions

Fifty milligrams each of standard SIL and HCT powder were accurately weighed and dissolved in a 100-mL mixture of methanol:water (1:1, *v/v*) by sonication for 10 min. The SIL standard solutions were diluted by the mixture of methanol:water (1:1, *v/v*) and phosphate buffer (pH 6.0) to obtain the required working range concentrations for HPLC and spectrofluorimetry, respectively. Each of the SIL standard solution contains 25 ppm HCT solution during HPLC analysis. The solutions were filtered through a 0.45 μ m membrane filter before injection into the HPLC system.

Preparation of Urorec[®] capsules

For both HPLC and spectrofluorimetry, ten Urorec[®] capsules were carefully weighed and powdered to get a homogenous fine powder in a mortar. An appropriate weight of this powder equivalent to one capsule content was weighed, transferred into the calibrated flask and then dissolved in the mixture of methanol:water (1:1, *v/v*) in an ultrasonic bath. The final mixture was filtered by the use of a 0.45 μ m membrane filter, and the filtrate was diluted with the mixture of methanol:water (1:1, *v/v*) and phosphate buffer solution (pH 6.0) to obtain the certain concentration in the linearity range of SIL for HPLC and spectrofluorimetry, respectively.

Forced degradation and stability-indicating tests

Ten milligrams each of standard SIL and HCT were dissolved to prepare the stock solution in a 25 mL mixture of methanol:water (1:1, *v/v*) by sonication for 10 min. After forced degradation process, each solution was filtered through a 0.20 μ m PTFE syringe filter before injection into the HPLC system

Acidic degradation

In the presence of IS, 2.5 mL of 1.0 M HCl was added to 7.5 mL stock solution, and the mixture was kept at 80 °C for 1 h under reflux, cooled and neutralized with 1.0 M HCl to pH 7.0. Then, 6.25 mL of the solution was made up to 25 mL with ultrapure water. Finally, the solution was filtered through a 0.20 μ m PTFE syringe filter.

Alkaline degradation

In the presence of IS, 2.5 mL of 1.0 M NaOH was added to 7.5 mL stock solution, and the mixture was kept at 80 °C for 1 h under reflux, cooled and neutralized with 1.0 M NaOH to pH 7.0. Then, 6.25 mL of the solution was made up to 25 mL with ultrapure water. Finally, the solution was filtered through 0.20- μ m PTFE syringe filter.

Table 1 System performance parameters obtained by HPLC for SIL

Parameters	HPLC	Reference value (Center for Drug Evaluation and Research (CDER) 1994)
Retention time, t_r	2.47	
Capacity factor, k'	0.789	$k' > 2.0$
Resolution, R_s	7.727	$R_s > 2.0$
Selectivity, α	6.771	$\alpha > 1.0$
Theoretical plates, N (plates/column)	5819	$N > 2000$
Tailing factor, T	1.11	$T \leq 2.0$

Oxidative degradation

In the presence of IS, 2.5 mL of 5 % H₂O₂ was added to 7.5 mL stock solution, and the mixture was kept at 80 °C for 1 h under reflux then cooled, and the volume of the mixture was made up to 25 mL with ultrapure water. Finally, the solution was filtered through 0.20- μ m PTFE syringe filter.

Thermal degradation

Ten milligrams each of SIL and HCT powders were kept at 80 °C for 24 h. After that, each powder was dissolved in a mixture of methanol:water (1:1, *v/v*). An aliquot of these solutions was dissolved to get a required solution consisting of 50 ppm SIL and 25 ppm HCT. The solution was filtered through 0.20- μ m PTFE syringe filter.

Results and discussion

Optimization of HPLC conditions

Firstly, HPLC conditions were optimized to obtain a desired peak with high purity and resolution. Therefore, the various parameters affecting the peak shape, retention

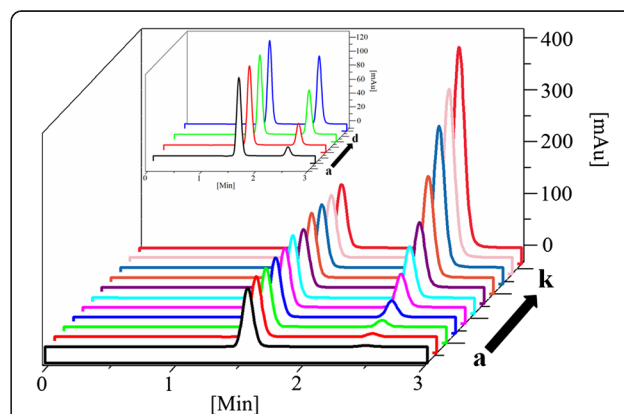


Fig. 2 HPLC chromatograms of a standard SIL solution at increasing concentrations in the range of 4.0 (a)–600.0 (k) μ M on a Agilent ZORBAX CN column using acetonitrile:methanol:acetate buffer (pH 4.0) (30:40:30, *v/v/v*) as a mobile phase at a flow rate of 1.3 mL min⁻¹ (Inset: the zoomed chromatograms at increasing SIL concentrations from 4.0 to 50.0 μ M)

Table 2 Validation results obtained from HPLC for the quantitative determination of SIL

Parameters	HPLC
Linearity range (μM) ^a	4.0–600.0
Slope ^b	5.8625
Intercept ^b	0.0050
Correlation coefficient (R^2)	0.9988
LOD (nM)	85.2
LOQ (nM)	284.0
Repeatability of peak area, (RSD %) ^c	0.12
Repeatability of peak area, (RSD %) ^d	0.19

^aAverage of three determinations^b $R_{pa} \text{ (a.u.)} = m C_{SL} \text{ (}\mu\text{M)} + b$ where C is the concentration and I_{FL} is the fluorescence intensities^cIntra-day measurements—each value is the mean of five experiments^dInter-day measurements—each value is the mean of five experiments

time and resolution of SIL were investigated in detail. The separation efficiency of Agilent ZORBAX CN column (150 mm \times 4.6 mm, 5 μm) was compared to the monolithic column (Supelco[®] C₁₈; 150 mm \times 4.6 mm, 5 μm) for the determination of SIL under the same conditions, and the proposed column was chosen for the further optimization parameters.

During our preliminary experiments, the series of aqueous mobile phases containing buffer solutions with the different pH values in combination with different organic modifiers including the different ratios of acetonitrile, methanol and ammonium acetate were tested for obtaining the optimum separation conditions. Acetonitrile, methanol and ammonium acetate were selected as the eluents. The chromatographic analysis time of SIL was shortened with high organic solvent content, and also, the buffer solutions in the mobile phase ensured stable chromatographic retention times preventing broad peaks.

The effect of the mobilephase pH on the retention time and peak shape of the analyte was studied especially in the acidic region. The best retention time and peak shape of SIL was achieved at pH 4.0 acetate buffer. The best separation was achieved with the mobile phase consisting of methanol:acetonitrile:acetate buffer (pH 4.0) (40:30:30, $v/v/v$). HCT was chosen as the IS as its retention time did not prolong the analysis time and indicated no interference effect on the chromatographic peak of SIL. The calibration curves of SIL for HPLC analysis were constructed by plotting the peak area ratio of drug molecule to IS against the concentration of the drug.

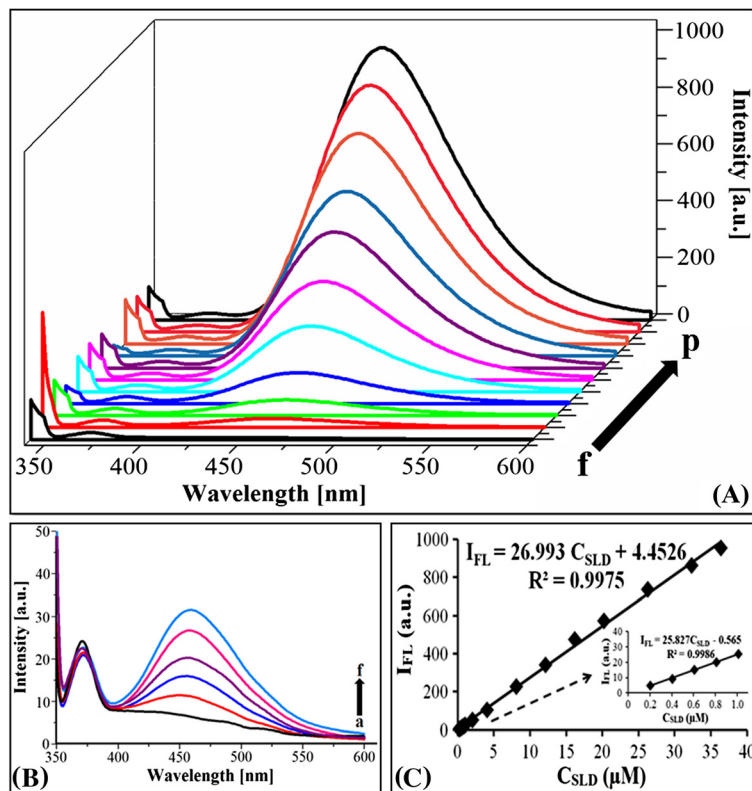


Fig. 3 Fluorescence spectra of SIL at increasing concentrations in the range of 0.2–36.4 μM at 460 nm after excitation of 330 nm in pH 6.0 phosphate buffer (the concentration range: 0.2 (b)–1.0 (f) μM (b); 1.0 (g)–36.4 (p) μM (a)). Fluorescence spectrum of blank solution ((a) and (f)) containing methanol:water: pH 6.0 phosphate buffer). The calibration curve of SIL obtained by spectrofluorimetry (c) in pH 6.0 phosphate buffer

Table 3 Validation results obtained from spectrofluorimetry for the quantitative determination of SIL

Parameters	Spectrofluorimetry
Wavelength (nm)	$\lambda_{\text{ex}} = 330$, $\lambda_{\text{em}} = 460$
Linearity range ^a (μM)	0.2–36.4
Slope ^b	26.993
Intercept ^b	4.4526
Correlation coefficient (R^2)	0.9975
LOD (nM)	7.70
LOQ (nM)	26.80
Repeatability of peak intensity (RSD %) ^c	0.48
Repeatability of peak intensity (RSD %) ^d	0.85

^aAverage of three determinations^b I_{FL} (a.u.) = $m C_{\text{SL}} (\mu\text{M}) + b$ where C is the concentration and I_{FL} is the fluorescence intensities^cIntra-day measurements—each value is the mean of five experiments^dInter-day measurements—each value is the mean of five experiments

Optimization of spectrofluorimetry conditions

In the second part of study, SIL exhibited the intense native fluorescence in phosphate buffer (pH 6.0) at 460 nm after an excitation of 330 nm as shown in Fig. 1. This feature gives a chance to develop a novel spectrofluorimetric method for determination of SIL in its pharmaceutical dosage forms. In this point, the various experimental parameters affecting the fluorescence intensity of SIL were carefully studied and the developed method was optimized in detail.

Effect of pH and excitation wavelength on fluorescence intensity

It is a known fact that the intensities of fluorescent molecules are connected with the pH value of the medium and excitation wavelength. The influence of pH on the fluorescence intensity of SIL was investigated using different pH values in the range from 2.0 to 10.0 with the three replicate measurements. The maximum fluorescence intensity of SIL was observed at pH 6.0 as shown in Fig. 1a. The effect of the excitation wavelength on

fluorescence intensity was also performed with three replicate measurements in the range of 310–360 nm. It was found that maximum fluorescence intensity of SIL was obtained at excitation wavelength of 330 nm in pH 6.0 phosphate buffer as shown in Fig. 1b.

Method validation

Chromatographic and spectrofluorimetric approaches have been useful techniques for the determination of drug or biologically important molecules in real samples for many years, and these techniques offer a simple way to quantify the drug molecules especially in pharmaceutical formulations (Belal et al. 2013; Antunes et al. 2013). Therefore, the presented study aims to develop validated analytical methods for the determination of SIL in pharmaceutical samples.

To evaluate the analytical performance of our proposed methods, the various analytical parameters (linearity, accuracy, precision, etc.) were investigated in detail under optimized conditions (International Conference on Harmonisation (ICH) 2005). The chromatographic analysis of SIL was successfully performed using the proposed method (Agilent ZORBAX CN column; 150 mm \times 4.6 mm \times 5 μm ; sample volume of 10 μL ; a mobile phase composed of acetonitrile, methanol and acetate buffer (pH 4.0); (30:40:30, v/v/v) at a flow rate of 1.3 mL min⁻¹; ambient temperature; detection wavelength of 270 nm). System suitability parameters were performed prior to analysis based on United States Pharmacopeia (USP) (The United States Pharmacopeia 2011). These parameters including tailing factor, peak resolution and selectivity, retention time and number of theoretical plates for the proposed method were evaluated, and the obtained values were in the acceptable ranges according to USP requirements as shown in Table 1 (Center for Drug Evaluation and Research (CDER) 1994).

Figure 2 indicates that peak area ratio (R_{pa}) of SIL was linearly increased with the SIL concentration in the range from 4.0 to 600.0 μM with a detection limit of

Table 4 The comparison of developed analytical methods used in determination of SIL

Method	Linearity range (μM)	LOD	Reference
UV	8.1–40.4	0.031 μM	(Jahan and Malipatil 2014b)
Spectrofluorimetry	0.02–2.0	6.1 nM	(Bhamre and Rajput 2014)
HPLC	20.2–60.5	6.16 μM	(Jahan and Malipatil 2014a)
RP-HPLC	20.2–121.0	11.00 μM	(Aneesh and Rajasekaran 2012)
HPLC (chiral)	0.26–9.1	80.7 nM	(Vali et al. 2012)
UHPLC	0.5–3.0	0.161 μM	(Shaik et al. 2014)
LC-MS/MS	0.001–0.1	0.30 nM	(Zhao et al. 2009)
GRP/AuNPs/GCE	0.01–3.3	3.80 nM	(Er et al. 2015)
Spectrofluorimetry	0.2–36.4	7.7 nM	Our study
RP-HPLC	4.0–600.0	85.2 nM	Our study

Table 5 Analysis of SIL in capsules using proposed methods

Parameters	Spectrofluorimetry	HPLC
Claimed (mg)	8.00	8.00
Found (mg)	7.85	7.96
RSD (%)	4.50	0.51
Average recovery (%)	98.50	99.50
RSD of recovery (%)	0.63	0.50
<i>t</i> value	2.69	
<i>F</i> value	1.51	

Critical value of *t* = 2.78 at 95 % confidence level (*n* = 5)Critical value of *F* = 6.39 at 95 % confidence level (*n*₁ = 5 and *n*₂ = 5)

85 nM (*S/N* = 3) under optimized conditions. The validation results obtained from HPLC are presented in Table 2. The related regression equation is as follows:

$$R_{pa} = 5.8625 C_{SIL}(\mu M) - 0.0050 \quad (R^2 = 0.9988) \quad (1)$$

SIL analysis was performed at 460 nm after excitation at 330 nm in pH 6.0 phosphate buffer for the proposed spectrofluorimetric method. The fluorescence signal at 370 nm was caused from a blank solution system consisting of methanol:water:phosphate buffer (pH 6.0) (10:10:80, *v/v/v*). The fluorescence intensity (*I*_{FL}) of SIL was observed to be a good linear relationship with the increasing concentration in the range of 0.2–36.4 μM as shown in Fig. 3. The regression equation for SIL is as follows:

$$I_{FL} = 26.993 C_{SIL}(\mu M) + 4.4526 \quad (R^2 = 0.9975) \quad (2)$$

The limit of detection (LOD) for SIL was found to be 7.7 nM calculated from related equation (*S/N* = 3). The similar study claimed that a narrow working range such as 0.1–3.0 μg mL⁻¹ for SIL are obtained at the excitation wavelength of 340 nm, and the best results for SIL were obtained at the excitation wavelength of 272 nm (Bhamre and Rajput 2014). Contrary to these results, the fluorescence intensity of SIL was gradually decreased with decreasing excitation wavelength from 330 to the less excitation wavelength in our study as shown in Fig. 1b. In this point, we presented a highly large linear range for SIL at the excitation wavelength of 330 nm in the buffer solution system.

The validation results including linearity, precision and accuracy of our proposed spectrofluorimetric method are presented in Table 3. The repeatability of results obtained by spectrofluorimetry for inter- and intra-day precisions was examined in the standard SIL solution at the same conditions. The relative standard deviation (RSD) values for inter- and intra-day precision were calculated to be 0.48 and 0.85 %, respectively. The results indicated that the proposed method has a highly satisfactory repeatability for the determination of SIL.

A comparison between the analytical parameters of the present methods and some previous methods reported in the literature for the determination of SIL is presented in Table 4. The proposed methods have sufficiently low LOD

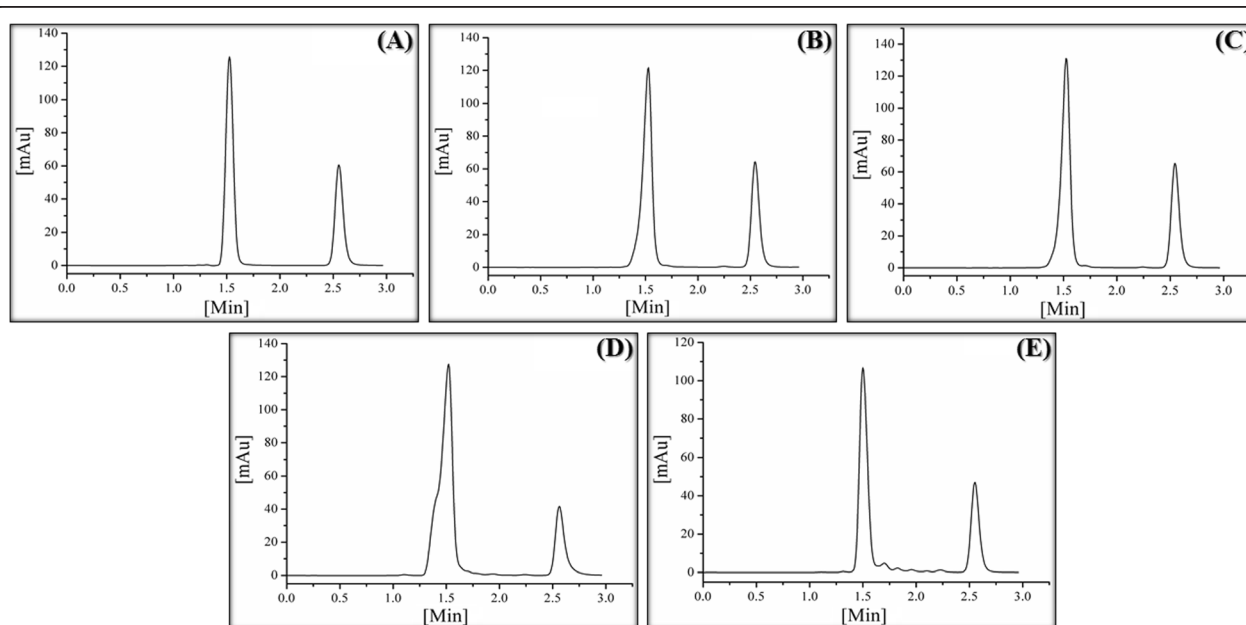
**Fig. 4** HPLC chromatograms of SIL in the presence of HCT (a), exposed to acidic (b), alkaline (c), oxidative (d) and thermal (e) degradations

Table 6 Results of forced degradation study of SIL drug using the developed stability-indicating HPLC method

Stress conditions	Heat temperature (°C)	Time (h)	SIL	
			Degradation (%)	RSD (%)
Acidic (1.0 M HCl)	80	1	11.98	0.32
Alkali (1.0 M NaOH)	80	1	11.83	0.17
Oxidative degradation (5 % H ₂ O ₂)	80	1	50.85	1.45
Thermal degradation	80	24	9.30	0.11

values compared to previously reported values for the detection of SIL.

Analytical applications

Silodosin assay in Urorec® capsules

Real-sample analysis is highly important to evaluate the system suitability of the proposed method towards the drug molecule with an acceptable recovery. Hence, pharmaceutical capsules (Urorec®) were selected as a real sample to show the applicability of our proposed methods. The results obtained from HPLC and spectrofluorimetry showed that the amount of SIL in capsules by both proposed methods is in good agreement with the labelled claim as summarized in Table 5. No interfering peaks were observed in the recorded chromatograms and fluorescence spectra indicating that there is no interference effect resulting from excipients used in the production of capsules and buffer medium. The recovery values were found to be 98.5 and 99.5 % for spectrofluorimetry and HPLC, respectively. The corresponding RSD values were well below 5.0 % indicating the proposed methods are sufficiently accurate and precise for quantitative purposes in analytical applications. Besides, statistical performance of the spectrofluorimetric results was compared with the results of the developed HPLC method using Student *t* and *F* tests at a 95 % confidence level as shown in Table 5.

Forced degradation behaviour

Forced degradation experiments were also performed to evaluate the stability and specificity of the proposed HPLC method in different mediums. The acidic, alkaline and oxidative degradation of SIL in the presence of IS were studied by treating with 1.0 M HCl/1.0 M NaOH/5 % H₂O₂ solutions at 80 °C for 1 h, respectively. The thermal degradation of SIL in the presence of IS were also studied by heating the SIL solution at 80 °C for 24 h. The whole degradation products were observed at approximately 1.50 and 2.50 min for HCT and SIL, respectively, in all proposed stress conditions as shown in Fig. 4. The stress studies showed no significant difference in terms of retention times of drugs, and no interfering peaks were observed within the retention time under alkaline, acidic, oxidative and thermal degradation conditions. Considering all these data, SIL was successfully

separated from all the degradation products which were confirmed by the resolution values calculated from each chromatogram ($R_s > 1.5$) (Table 6).

Conclusions

A highly sensitive and effective validated reversed-phase HPLC method was successfully developed with a low LOD value for SIL assay. The SIL in the presence of HCT were subjected to forced degradation under several stress conditions. The satisfactory results were achieved from degradation studies, which revealed that the method was stability indicating. Besides, the spectrofluorimetric approach has drawn attention with satisfactory results in the detection of SIL in a pharmaceutical sample. The proposed methods have significant advantages such as rapid, sensitive, not needing a fluorescent agent and derivatization reactions, good reproducibility and cost effectiveness. The results showed that the developed analytical methods could be a very promising alternative for the determination of SIL in routine drug and clinical application.

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Authors' contributions

Both authors equally contributed in experimental design, analysis results, writing and proofing and approved the final manuscript. Experimental work was performed by EE.

Competing interests

The authors declare that they have no competing interests.

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